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THE EFFECT OF VARIOUS HYDROXYLATED
TRYPTAMINES ON CNS MONOAMINE –
– CONTAINING NEURONS IN THE RAT

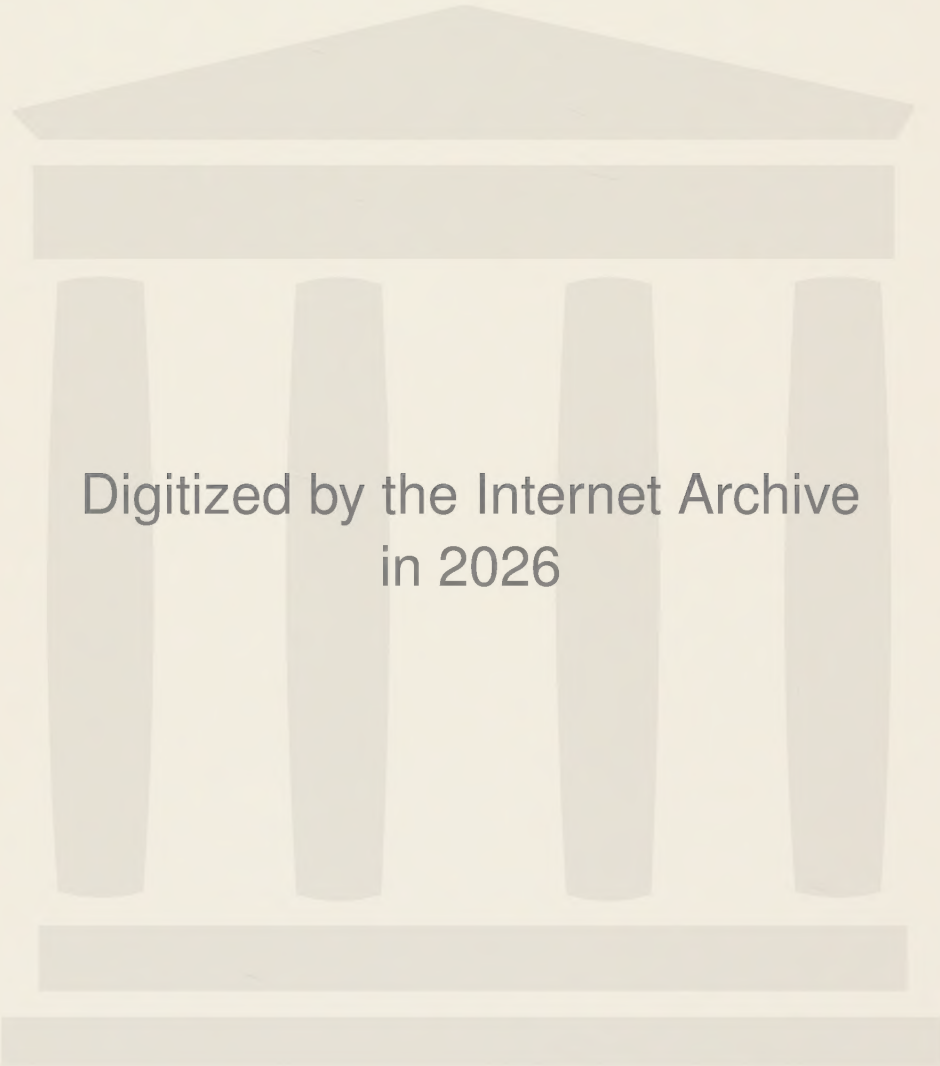
BY

HANS GEORG BAUMGARTEN

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MONOAMINE-CONTAINING NEURONS IN THE RAT

AKADEMISK AVHANDLING

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HANS GEORG BAUMGARTEN
Sm.

From the Institute of Anatomy and Histology, University
of Lund, Lund, Sweden, and from the Department of Neuro-
anatomy, University of Hamburg, Hamburg, G.F.R.

THE EFFECT OF VARIOUS HYDROXYLATED TRYPTAMINES ON CNS MONO-
AMINE-CONTAINING NEURONS IN THE RAT

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HANS GEORG BAUMGARTEN

LUND 1973

The present thesis is based on the following papers:

- I. Long-lasting selective depletion of brain serotonin by 5,6-dihydroxytryptamine. Acta physiol.scand. Suppl. 373(1971)1-15 (together with A.Björklund, L.Lachenmayer, A.Nobin and U.Stenevi).
- II. Effects of 5,6-dihydroxytryptamine on monoaminergic neurones in the central nervous system of the rat. J.Neurochem. 19(1972)1587-1597 (together with K.D. Evetts, R.B.Holman, L.L.Iversen, M.Vogt and G.Wilson).
- III. Chemical degeneration of indolamine axons in rat brain by 5,6-dihydroxytryptamine. Z.Zellforsch. 129 (1972)256-271 (together with A.Björklund, A.F.Holstein and A.Nobin).
- IV. Long-term recovery of serotonin concentrations in the rat CNS following 5,6-dihydroxytryptamine. Life Sci. Vol.12, I(1973)357-364 (together with L. Lachenmayer, A.Björklund, A. Nobin and E.Rosengren).
- V. Axonal degeneration and regeneration of the bulbo-spinal indolamine neurons after 5,6-dihydroxytryptamine treatment. Brain Res. 56(1973)1-24 (together with A.Nobin, A.Björklund, L.Lachenmayer and U.Stenevi).
- VI. Evaluation of the effects of 5,7-dihydroxytryptamine on serotonin and catecholamine neurons in the rat CNS. Acta physiol.scand. Suppl. 391(1973)1-19 (together with A.Björklund, L.Lachenmayer and A.Nobin).
- VII. Effect of intraventricular injection of 5,7-dihydroxytryptamine on regional tryptophan hydroxylase of rat brain. J.Neurochem. 21(1973)251-253 (together with S.J.Victor and W.Lovenberg).

VIII. Inhibition of the uptake of 5-hydroxytryptamine, noradrenaline and dopamine into rat brain homogenates by various hydroxylated tryptamines.

J.Neurochem.21(1973)233-236 (together with A.S.Horn and H.G.Schlossberger).

References to these papers will be made by citation of the appropriate Roman numerals.

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1 INTRODUCTION

The development of a sensitive and specific method for the fluorescence microscopical detection of certain biogenic monoamines in tissues by Falck and Hillarp (see Falck 1962; Falck, Hillarp, Thieme and Torp 1962; Corrodi and Hillarp 1963, 1964) was the prerequisite for the cellular demonstration of catecholamines and 5-hydroxytryptamine (5-HT) in nervous tissue and has dramatically enriched our understanding of the anatomy and physiology of monoaminergic neuron systems. The Falck-Hillarp method which is based on the formation of fluorescent derivatives of catechol- and indoleamines in freeze-dried tissue by formaldehyde gas condensation is, however, less sensitive for indoleamines than for biogenic catecholamines (see Falck 1962; Fuxe 1964; Björklund, Falck and Owman 1972). This lower sensitivity of the Falck-Hillarp technique for the demonstration of tryptamines is mainly responsible for the scanty present-day information on the anatomy and physiology of central (and possible peripheral) indoleamine-containing neurons compared to the detailed knowledge available on the distribution and projections of dopaminergic and noradrenergic neurons in relevant literature. This is mainly due to the fact that the molar fluorescence yield of the condensation reaction between e.g. serotonin (5-HT) and formaldehyde is lower than that for catecholamines, that the fluorophore formed from serotonin and formaldehyde is rapidly decomposed when irradiated with ultraviolet or blue excitation light (Falck 1962; Caspersson, Hillarp and Ritzen 1966; Ritzen 1966), and that many of the varicosities of central 5-HT neurons are probably rather small in diameter and perhaps at the border of the resolution power of the fluorescence microscope (Fuxe 1964).

The situation is even more uncertain at the ultrastructural level, since with conventional methods of fixation giving satisfactory preservation of the ultramorphology (i.e. glutaraldehyde and osmium), serotonin synapses are indistinguishable from catecholamine-containing ones and both are difficult to differentiate from non-monoaminergic synapses.

Techniques employing mechanical or electrolytic interruption of axonal pathways are in the CNS complicated by the simultaneous damage of other neuron types releasing different transmitters, by disturbances of local blood supply, and by extended glial reactions (scar tissue formation). The results gained from such experiments are questionable what concerns the selectivity of interruption of homogeneous populations of neurons and axons and this is particularly evident in case of the diffusely organized monoamine neuron systems. The development of neurotoxic drugs capable of selectively destroying serotonin synapses, axons and eventually cell bodies was, therefore, supposed to assist in improving our knowledge on indoleamine projections and functions in the mammalian brain.

In analogy with 6-hydroxydopamine (6-OH-DA), which selectively destroys catecholamine-containing neurons in brain and peripheral nervous system, it was thought reasonable to search for dihydroxyindoleamines for lesioning brain serotonin neurons. From all the potentially neurotoxic hydroxyindoleamines (4,5-;5,6-;6,7-;4,6-;4,7- and 5,7-dihydroxytryptamine ; 4,5-DHT,5,6-DHT,6,7-DHT,4,6-DHT,4,7-DHT and 5,7-DHT) those sharing with serotonin the hydroxyl group in position 5 of the indole nucleus were supposed to be the most selective compounds for this purpose (4,5-; 5,6- and 5,7-DHT) since they share with serotonin the 5 hydroxyl group which is probably important for selective uptake of indoleamines into 5-HT neurons. Among these,

5,6-dihydroxytryptamine was hypothesized to be the most suitable for the induction of selective disintegration of 5-HT neurons, since 6-hydroxytryptamine (6-HT) had earlier been shown to have a good affinity to the membrane-bound monoamine uptake system of 5-HT neurons (Jonsson, Fuxe, Hamberger and Hökfelt 1969) and would thus presumably not severely interfere with the uptake properties of the 5-hydroxyl group in the 5,6-DHT molecule. Furthermore, hydroxylation of tryptamine at position 4 and 7 has not been demonstrated to occur in mammalian tissue, but 6-hydroxylation of tryptamine (and melatonin) represents a possible catabolic principle in certain mammalian tissues (e.g. the liver, see Wurtman, Axelrod and Kelly 1968; Lemberger, Axelrod and Kopin 1971). Finally, according to the theory advanced by Saner and Thoenen (1971) and Blank et al. (1972) to explain the mechanism of action of 6-hydroxydopamine on catecholamine neurons, 5,6-dihydroxyindoles - formed from oxidation and cyclization of 6-OH-DA in tissues - are probably important for the neurotoxic properties of this catecholamine analogue. If so, 5,6-dihydroxytryptamine should be a very potent and selective compound for the induction of degeneration in central serotonin neurons.

2 MATERIAL

For all studies adult male or female albino Wistar rats (180-220 g body weight at the time of drug injection) were used, except for the investigation on de- and regeneration of bulbo-spinal serotonin neurons (Paper V) where albino rats of the Sprague Dawley strain were used.

Drugs: 6-hydroxytryptamine-creatinine sulfate (through Prof.A.Hürlimann,Hoffmann-La Roche), 5,6-dihydroxytryptamine-creatininesulfate (through Dr.H.G.Schlossberger, Munich, or from Regis Chemical Co.), 4,5-dihydroxytryptamine-creatinine sulfate and 4-hydroxy-5-methoxy-tryptamine HCl (through Dr.H.G.Schlossberger, Munich), 7-hydroxytryptamine-creatinine sulfate and 5,7-dihydroxytryptamine-creatinine sulfate (through Dr.A.Manian, N.I.M.H.,Bethesda),alpha-methyl-m-tyrosine (AB Hässle, Göteborg), nialamide (Niamid^R, Pfizer), Brietal^R (Lilly), sodium pentobarbitone (Nembutal^R Abbott).

3 METHODS

3.1 Histochemistry of monoamines (Papers V,VI,VIII)

The formaldehyde condensation of Falck and Hillarp (as described in detail by Falck and Owman 1965, Björklund, Falck and Owman 1972 and Baumgarten 1972) was employed to visualize tissue monoamines fluorescence microscopically. In order to selectively demonstrate indoleamines in tissues and to abolish interfering catecholamine fluorescence, some animals were pretreated with alpha-methyl-m-tyrosine and nialamide (see Paper V), or nialamide alone (see Paper VI). The specificity of the fluorescence phenomena seen in freeze dried tissues after formaldehyde treatment was controlled by applying the sodium borohydride test to tissue sections (Corrodi, Hillarp and Jonsson 1964).

3.2 Microspectrofluorometry (Paper V)

Spectral analysis of amine fluorophores in tissue sections was carried out with a modified Leitz microspectrofluorimeter (Björklund, Ehinger and Falck 1968, 1972). Only corrected values are reported. The correction of the instrument values was performed according to the device by Ritzen (1967), as applied by Björklund, Falck and Owman 1972 and Björklund, Ehinger and Falck 1972.

3.3 Electron microscopy (Paper III)

Tissues for electron microscopical analysis were obtained from animals prepared by perfusion fixation with cold (8-12°C) glutaraldehyde (6% in 0,05 M phosphate buffer at pH 7,2) via the cannulated aortic trunk from the left cardiac ventricle in deep nembutal anaesthesia (60 mg/kg ip.). Postfixation of aldehyde prefixed small tissue blocks was performed in cold (4°C) phosphate buffered (0,1 M) osmium tetroxide (1%) solution (containing 0,1 M sucrose) at pH 7,2 for 2 hrs. For

dehydration and embedding the procedure described by Luft (1961) was applied. Tissue sections were prepared on a Reichert ultramicrotome OmU 2 and electron micrographs were taken in a Philips EM 300.

3.4 Chemical determinations of monoamine concentrations and enzyme activity (Papers I,II,IV,V,VI,VII)

5-HT was estimated fluorimetrically according to the method of Bertler (1961)(Papers I,IV,V,VI), according to a combination of the method by Bertler (1961) and Maickel,Cox,Saillant and Miller (1968) (Paper II), and 5-HT and 5-hydroxy indole acetic acid (5-HIAA) according to a combination of the procedures described by Bertler (1961) and Contractor (1966) (Paper II). Noradrenaline (NA) and dopamine (DA) were determined according to Bertler, Carlsson, Rosengren and Waldeck (1958) as modified by Häggendal (1963) (Papers I,IV,V,VI) or NA according to the device by Euler and Lishajko (1961) and DA by the method of Carlsson and Waldeck (1958) (Paper II). Monoamine concentrations given in Papers I,II,IV,V,VI were not corrected for recovery. Tyrosine hydroxylase activity in homogenates of different brain regions was estimated by the radiochemical method of Iversen and Uretsky (1970) (Paper II), tryptophane hydroxylase by a modification of the method of Friedman,Kappelman and Kaufman (1972) as described by Baumgarten,Victor and Lovenberg (Paper VII).

3.5 Inhibition of amine uptake by hydroxyindole compounds in vitro (Papers II and VIII).

In order to obtain information on the relative affinities of different hydroxyindoleamines for monoamine uptake sites in brain, the effect of 1×10^{-7} or

1×10^{-6} M 6-HT, 7-HT, 4,5-DHT, 4-hydroxy-5-methoxy-tryptamine, 5,6-DHT and 5,7-DHT on the uptake of ^3H 5-HT and ^3H NA into synaptosomes from homogenates of rat hypothalamus and of ^3H DA uptake into synaptosomes from rat striatum was analyzed according to the method of Horn and Snyder (1972) (Paper VIII) or according to the method of Iversen and Neal (1968) and Iversen (1970) (Paper II) on incubated slices from rat striatum and hypothalamus.

3.6 Uptake of tritiated monoamines by brain and spinal cord slices in vitro (Papers II and VI)

The uptake of labelled monoamines into incubated slices from rat cortex, hypothalamus and spinal cord following pretreatment of the animals with 5,7-DHT (9-11 days after drug injection into the lateral ventricle) was determined according to the procedure described by Björklund, Nobin and Stenevi (1973) (Paper VI) and the uptake of ^3H 5-HT into slices from rat cortex, hypothalamus and striatum 4 days after the administration of 5,6-DHT by the method of Iversen (1970) and Iversen and Neal (1968) (Paper II).

3.7 Injections of monoamines into the brain ventricles (Papers I-VIII)

Injections of monoamines into the left (or right) lateral ventricle of rat brain were performed according to the method of Wurtman, Noble and Axelrod (1967), using 20 μl sterile 0,9% NaCl solution as vehicle for each injection. The vehicle contained 0,1-1,0 mg/ml ascorbic acid in order to prevent oxidation of the indole compounds. Hamilton microliter syringes were used for the intraventricular injections.

4 RESULTS AND COMMENTS

4.1 Effect of 5,6-dihydroxytryptamine on CNS monoamine content in the rat (Papers I,II,IV)

The effect of different doses of 5,6-DHT (25,50 and 75/ μ g) on the CNS 5-HT concentration was analyzed in six selected brain regions and in the spinal cord of the rat, 10 days after the intraventricular application of the drug (Paper I). All doses tested caused a highly significant drop of 5-HT in septum, hypothalamus, forebrain rest sample (after removal of the septum, striatum and hypothalamus), mesencephalon and spinal cord. The loss of 5-HT from the pons/medulla oblongata was less pronounced. 25/ μ g 5,6-DHT had no significant effect on the striatal 5-HT, but 50 and 75/ μ g 5,6-DHT caused a significant depletion also in this structure. The depletion, e.g. following 75/ μ g 5,6-DHT, varied considerably between different regions. In some brain regions (mesencephalon, hypothalamus, septum, forebrain rest) the extent of 5-HT depletion at 10 days ranged in between 40 and 60%. The loss of 5-HT from the striatum and pons/medulla oblongata was markedly lower (10-30%). The greatest reduction in 5-HT was found in the spinal cord (85%). A dose response effect was unraveled only in some brain regions (hypothalamus, septum, striatum and forebrain rest sample), i.e. those regions comprising reasonable amounts of ventricle-near or surface-near structures which are primarily exposed to the drug injected into the CSF. In the remaining regions (the cell body rich parts pons/medulla oblongata and mesencephalon) and the spinal cord (with ventricle-distant 5-HT terminal systems grey matter) no dose response effect was evident (see below and Paper V).

The results point to the necessity of analyzing the effect of neurotoxic drugs in subdivided regions of the central nervous system since evidently the mechanism of action of the drug is not the same in different parts of the brain or spinal cord. The validity of this statement is clearly supported by the results of a detailed study on the time course effects of 75 μ g 5,6-DHT on regional 5-HT concentrations (Paper I).

Very distinct and different patterns of 5-HT depletion and recovery were unraveled in different regions:

- 1) A simple pattern of depletion was found in the spinal cord, characterized by gradual slow depletion of 5-HT which was nearly complete at 4-5 days after 5,6-DHT. This protracted, slow depletion of transmitter essentially conforms to the time course of loss of 5-HT or tryptophan hydroxylase from the spinal cord after a high cervical transection (Carlsson, Falck, Fuxe and Hillarp 1964; Clineschmidt, Pierce and Lovenberg 1971; Anderson 1972) and can be interpreted as signifying anterograde disintegration of terminal systems due to a primary damage of their parent non-terminal axons by the drug. This interpretation gains support from the results of a fluorescence microscopical study carried out on the bulbo-spinal 5-HT neuron system following the application of 25 or 75 μ g 5,6-DHT (Paper V), showing that the 5-HT terminal systems in the spinal cord are not directly affected by the drug.
- 2) In the ventricle-near parts of the brain (septum, striatum, hypothalamus and mesencephalon) 5-HT was rapidly depleted to minimum levels at 12 hrs. The values then persisted at levels close to the early minimum at least up to the 16th day. A slight but significant recovery in 5-HT was noticed in between the 16th and 30th day in the hypothalamus, mesencepha-

lon and septum.

3) The pons/medulla oblongata differed in its response from that of the ventricle-near brain regions by an early, partial but significant recovery phase between 12 hrs and 4 days. This difference in the time course pattern of 5-HT depletion may be explained by assuming that 5,6-DHT affects cell bodies of serotonin neurons only transiently (as supported by the morphological data, see below and Paper V) and by assuming that the cell bodies react to the drug-induced damage of their terminals by an increased synthesis and down-transport of transmitter synthesizing enzymes, above all tryptophan hydroxylase. Victor, Baumgarten and Lovenberg (1973) have shown that the activity of tryptophan hydroxylase in the pons/medulla oblongata sample rises to levels significantly above control by the 4th day after the application of 75 μ g 5,6-DHT. Furthermore, there are large accumulations of serotonin in dilated and distorted indoleamine axons during this time period especially in the pons/medulla oblongata (see below and Paper V). At one month, serotonin levels in this region of the rat CNS no longer differed significantly from control.

In the time course study referred to (Paper I), possible asymmetries of the effect of the drug injected into one of the two lateral ventricles have been disregarded. This problem has been investigated in another chemical study on the effects of 5,6-DHT on rat brain monoaminergic neurons (Paper II). It was found that the degree of depletion of 5-HT in ventricle-near regions in the rat (measured in caudates, septa and hypothalamus pooled from single rats) was consistently greater on the injected than on the non-injected side. This difference persisted throughout the time investigated (up to 2 months after 5,6-DHT).

In addition, two other parameters, namely the speed of injection of the compound and the type of anaesthesia used, proved to be of importance for the degree of depletion of 5-HT. Thus, rapid injection of 5,6-DHT under ether caused a greater depletion of 5-HT and 5-HIAA than slow injection of the compound under pentobarbitone. Under ether, even a slow administration of 5,6-DHT produced a greater loss of 5-HT from the pons/medulla oblongata than under pentobarbitone, and was even more effective than rapid injection under ether.

Another important observation made in this study was that 5,6-DHT caused, besides selective effects on central nervous serotonin content, also unselective toxic damage to brain structures located close to the injection cannula, i.e. a significant atrophy of the caudate and septum of the injected side as compared to the non-injected side. Careful electron microscopical investigations on the caudate and septum of the injected side of the brain revealed damage to ventricle-near myelinated fibre systems (cf. Paper III).

In these investigations, another finding of general interest and significance was made. The effect of 5,6-DHT on the serotonin content of the pons/medulla oblongata was measured with two different techniques, one technique using the native fluorescence of indoles in acid solution following amberlite separation of the extracts and the other technique measuring the fluorescence of condensation productus from o-phthalaldehyde and indoleamines after the same purification. Invariably, the loss of indoleamines was greater with the o-phthalaldehyde method (60% depletion with the o-phthalaldehyde versus 10-20% with the conventional acid fluorescence method). These differences suggest that the assay methods have different sensitivities and specificities for different indole derivatives. This observa-

tion which calls for criticism against the specificity of some of the 5-HT assay procedures (especially the condensation reactions) seems, however, relevant to Björklund's and co-workers' recent discovery of two different types of indoleamine fluorophores in the rat brain using microspectrofluorometry (Björklund, Falck and Stenevi 1971 a,b. Support for this assumption comes from the recent detection of 5-methoxytryptamine as a regular constituent of the rat hypothalamus (Green, Koslow and Costa 1973). It is particularly interesting in this context that the 5,6-DHT did not seem to cause any damage to these fibre systems storing a non-serotonin indolealkylamine (Paper V, Björklund, Nobin and Stenevi 1973); perhaps 5,6-DHT can be used for selectively demonstrating this second indoleamine neuron system by degenerating the interfering serotonin axons and terminals.

Another important aspect of the action of neurotoxic drugs like 5,6-DHT is their specificity against defined monoamine neuron systems. At least chemically (Paper I and II) there was no consistent long-term effect of 50 μ g 5,6-DHT on either CNS NA or DA concentrations. With 75 μ g 5,6-DHT, dopamine was initially depleted but returned to near-normal values by 5 days after the injection, whereas noradrenaline showed a transient elevation above control levels between 4 and 30 days (Paper I). A slight but significant reduction in forebrain NA and striatal DA concentrations was recorded 4 days after 100 μ g 5,6-DHT (Paper II), indicating that 5,6-DHT when given in doses not higher than 75 μ g per injection, rather selectively affects CNS indoleamine concentrations.

4.2 Electron microscopical evidence for degeneration of indoleamine terminals and axons and unspecific toxic effects following the application of 5,6-DHT (Paper III)

Although the long-term depletion and the consecutive, slow, gradual recovery of CNS serotonin concentrations in brain after 5,6-DHT suggest that the drug acts by inducing degeneration of 5-HT terminals - as does the disappearance of serotonin fluorescence in ventricle-near terminal systems - the final proof for the potential selective neurotoxicity of this compound can only be provided by electron microscopy. The short-term effects of one single (50 or 75 μ g) or two consecutive intraventricular doses of 5,6-DHT (given 24 hrs apart) were analyzed electron microscopically in brain regions known to receive a substantial amount of serotonin projections (e.g. the anterior and middle periventricular hypothalamus, the subcommissural organ) , and, for comparison, regions known to have an additional supply of noradrenergic (septum) and dopaminergic fibres (head of the left and right caudate nucleus). Discrete changes in terminal (synaptic) and non-terminal varicose swellings of unmyelinated axons developed as early as 2-6 hrs after drug administration and were mainly characterized by conspicuous clumping and enlargements of vesicles, hypertrophy of tubular profiles and a disorganization of the ultrastructure of the axoplasm. Later changes (24-48 hrs) comprised dissolution of vesicles, electron dense impregnation of mitochondria and of the axoplasm, formation of multilamellar inclusion bodies (cellular autophagia), and, finally, incorporation of the dystrophic remnants of axons into glial cell cytoplasm. In addition,

non-terminal axons were found to exhibit the classical signs of fibrillar dark degeneration. Beginning at 24 hrs, increasing numbers of large axonal swellings were detected that correspond to the distended, stump-like, highly fluorescent indoleamine axons. In all their varying ultramorphological features, they correspond to proximal stumps of mechanically severed monoamine axons, e.g. noradrenaline-containing axons of peripheral nerves after compression or ligature (Kapeller and Mayor 1967).

Besides these drug-induced changes - which may be interpreted as indicating more or less selective lesions and reactions of presumptive indoleamine-containing axons - also toxic damage to non-monoaminergic structures, as e.g. myelinated fibres, were observed. Toxic myelin damage and even degeneration of myelinated axons were unraveled in the head of the caudate nucleus and the opposing septum, especially of the injected side. These findings clearly show that 5,6-DHT is complicated by a potential unselective toxicity, and, that neurotoxic drugs of this kind might all be complicated by varying degrees of general tissue toxicity. This is not surprising in view of the chemical properties of 5,6-DHT, which, at biological pH, is rapidly oxidized to its corresponding ortho-quinone. 5,6-ortho-indolequinone by all probability is provided with a high chemical reactivity, giving rise to different substitution or addition reactions with low or high molecular compounds. This can be illustrated by e.g. injecting solutions of oxidized 5,6-DHT into the brain ventricles of rats which then respond by severe convulsion and death after a few minutes (Baumgarten and Lachenmayer, unpublished observations) while rats having received a comparable dose of unoxidized 5,6-DHT do not show any such symptoms, provided the dose is kept below

the limit of 75 μ g per animal. It is then of great importance that as much unmetabolized 5,6-DHT as possible reaches specific uptake sites in brain before undergoing autooxidation. Evidently, this is not the case with 50 or 75 μ g 5,6-DHT, since precipitation of a brownish, probably melanin-like, pigment is observed on the ventricular surface and bordering brain tissue suggesting that oxidation and polymerization must have occurred while the drug penetrated brain matter. It is interesting in this context that also 6-OH-DA may be complicated a certain degree of unselective toxicity when given in high amounts to experimental animals (see Poirier et al. 1972). Especially when used for physiological studies possible unspecific toxic side effects of all potentially neurotoxic drugs have to be carefully explored and controlled.

4.3 Chemical evidence for regeneration of serotonin neurons after 5,6-dihydroxytryptamine treatment (Papers I,II,IV)

Both studies (Paper I and II) dealing with the short-term effects of 5,6-DHT on CNS serotonin levels indicated a late phase of incomplete recovery of serotonin in all brain regions in between 16 days and 1 month, except the spinal cord. Assuming that the depletion of serotonin in between 1 and 16 days was mainly due to drug-induced direct toxic terminal and protracted indirect anterograde terminal degeneration of 5-HT axons, such a late rise of serotonin in brain could be interpreted as signifying regeneration of part of the drug lesioned 5-HT neurons. It was therefore of interest to see whether the tendency towards increased serotonin concentrations con-

tinued during the following months or whether it was only transitory. The results of the long-term recovery study using 75 μ g 5,6-DHT (see Paper IV) revealed a progressive and continuing increase of 5-HT in all brain regions investigated (septum, striatum, hypothalamus, mesencephalon, forebrain rest and pons/medulla oblongata) in between 1 and 6 months after drug application.

By 6 months, 5-Ht levels in the striatum, mesencephalon and forebrain rest sample no longer differed significantly from control values whereas those in the remaining brain regions had climbed to levels significantly above control (septum 160%, hypothalamus 135%, pons/medulla oblongata 120% of control). Contrary to this, the spinal cord remained depleted over the time period studied (15% of control in between 5 days and 6 months). Since fluorescence microscopically an extensive regrowth of drug-damaged indoleamine axons was observed in the hypothalamus, mesencephalon and within the lower medulla oblongata (Björklund, Nobin and Stenevi 1973; Paper V) in between 10 days and 6 months, the conclusion is justified that the recovery of serotonin concentrations in brain following 5,6-DHT induced axonal damage is due to regenerative sprouting. These findings prove that central serotonin neurons are capable of compensating for a discrete chemical injury by vigorous sprouting of their axons, similar to their ability to regenerative growth after mechanical interruption of their axonal pathways. While properly directed growth does not occur following a mechanical lesion, it does occur after a selective chemical damage (Paper V, Björklund, Nobin and Stenevi 1973).

Although entirely speculative at the present state of knowledge, this ability of central monoamine neurons to partially compensate for a toxic disintegration of their terminals and axons by regeneration may have some importance for the spontaneous recovery of brain function in humans following a long-lasting psychosis, on the event that the psychic disturbance was caused by a toxic degeneration of functionally meaningful monoamine terminals, such as hypothesized for schizophrenia by Stein and Wise (1971). On the other hand, the late recovery of brain function following traumatic injury (e.g. reappearance of consciousness in patients after a long-lasting comatose state) may also be connected to regrowth of traumatically severed monoamine axons, supposed to be involved in cortical arousal mechanisms (such as e.g. the NA neuron system).

4.4 Morphological evidence for de- and regeneration of serotonin axons after 5,6-dihydroxytryptamine treatment (Paper V)

The results from chemical investigations suggested that de- and regeneration of CNS indoleamine neurons occurred after 5,6-DHT. The assumption that depletion and recovery of 5-HT following 5,6-DHT treatment are indeed due to de- and regeneration processes, was further substantiated in a fluorescence microscopical investigation on the bulbo-spinal serotonin neuron system of the rat (Paper V).

At either dose injected (25 or 75 μ g) 5,6-DHT provoked, by 12-24 hrs, the appearance of strongly fluorescent dilatations in the bulbo-spinal indoleamine neuron system which were mainly confined to the junction of the medulla oblongata and the spinal cord

and persisted nearly unchanged during the next 4 days. By 24 hrs most indoleamine-containing terminals in the grey matter of the spinal cord appeared unaffected, especially following the lower of 5,6-DHT. In between 2 and 10 days the indoleamine terminals progressively lost their formaldehyde-induced fluorescence. After 10 days, practically all regions of the spinal cord had lost their indoleamine innervation, whereas the yellow fluorescent terminals located in certain portions of the accessory olivary complex and the indoleamine cell bodies in the medulla oblongata seemed practically unaltered by the drug during the first 10 days. In animals with a survival time of 1-3 months, there was a loss of part of the indoleamine pericarya, particularly those located very superficially in the caudal medulla oblongata, in all probability reflecting a retrograde degeneration process due to a primary damage of their parent axons close to the cell bodies. These findings permit the conclusion that, in the bulbo-spinal serotonin system, 5,6-DHT mainly affects the non-terminal axons directly, and, that the disintegration of most of the 5-HT terminals is due to a secondary anterograde degeneration process, in harmony with the interpretation advanced for the slow protracted drop in the 5-HT levels recorded in the spinal cord after 75 μ g 5,6-DHT (Paper I).

Apart from retrograde degeneration of some of the most superficially located indoleamine neurons, cell bodies are generally less sensitive to direct toxic effects of the drug than terminals and non-terminal axons. This differential drug sensitivity and the fact that - by intraventricular application of 5,6-DHT - most axons are evidently not lesioned too close to

the pericarya seems to permit most of the indoleamine neurons to respond to the axonal injury by regenerative sprouting.

10 days, if not earlier, after 5,6-DHT, newly formed axonal sprouts were seen to develop close or even continuous to the swollen, distorted, stump-like indoleamine axons, invading the surrounding tissue. During the following weeks, increasing numbers of growing fibres were observed in many places of the medulla oblongata and the most cervical part of the spinal cord. By 3 months, newly established terminal networks of indoleamine neurons were found in e.g. the anterior horn of the cranial segments of the spinal cord (normally innervated by indoleamine terminals) or in the lateral reticular nucleus of the caudal medulla oblongata and the medial accessory olivary nucleus (hardly receiving any indoleamine innervation in control specimens). This suggests that besides re-innervation of drug-denervated areas in the rat CNS by sprouting indoleamine axons, also new establishment of connexions occurs during regeneration which indicates aberrant axonal growth in the adult brain.

4.5 The effects of 5,7-dihydroxytryptamine on central monoaminergic neurons in the rat (Papers VI,VII,VIII)

The detailed chemical and morphological work on the effects of 5,6-DHT on monoaminergic and non-monoaminergic structures of rat brain and spinal cord (Papers I-V) revealed definite limitations in the applicability of 5,6-DHT as a tool for the induction of serotonin degeneration. Firstly, 5,6-DHT was found to be selective for 5-HT neurons only in a restricted dose range (10-75 μ g). Secondly, doses higher than 75 μ g caused damage to central NA and DA neurons in addition to 5-HT ones and were severely toxic, provoking convulsions, limb paralysis and tremor in the pretreated

animals (Paper II and unpublished observations). Third, all doses of 5,6-DHT tested (25-100 μ g) induced moderate to severe damage to myelinated axons, either confined to the brain regions surrounding the tip of the injection cannula (all doses) or to surface-near myelinated tracts (e.g. the pyramidal tract and decussation of the ventral aspect of the medulla oblongata, with 75 or 100 μ g; see Paper V). Finally, part of the drug was found to be prevented from reaching specific uptake sites in brain due to a rapid conversion of 5,6-DHT into a brownish pigment soon after its intraventricular injection (Paper II). These observations prompted us to test other dihydroxyindoles for their potential neurotoxicity against CNS monoamine neurons that might not be complicated by the drawbacks noted with 5,6-DHT. In addition, several monohydroxytryptamines were investigated with the same methods in order to clarify whether or not two hydroxyl substituents on the benzene nucleus of the indole molecule are required for toxicity against CNS monoamine neurons.

Of all the compounds tested (see Paper VII), 5,7-DHT appeared the most promising for serotonin degeneration since it shares with serotonin the 5 hydroxyl group (which should provide 5,7-DHT with good affinity to the 5-HT uptake sites) and since it is, by its molecular structure, much less prone to autoxidation than 5,6-DHT.

Two different studies were carried out to evaluate the action of this meta-substituted dihydroxytryptamine on CNS monoamine-containing neurons: a combined chemical and morphological investigation (Paper III) and an enzymological study using tryptophan hydroxylase as a marker enzyme for serotonergic neurons (Paper IV).

Doses of 10-200 μ g 5,7-DHT applied to the ventricles of rats induced a dose-dependent long-lasting reduction of serotonin and noradrenaline in whole brain and spinal cord. With increasing doses up to 50 μ g 5,7-DHT, brain NA and 5-HT levels were equally depleted at 10 days after drug application (approximately 50% reduction of either amine). At higher doses of 5,7-DHT (75-200 μ g) serotonin was further reduced (up to 75% depletion after 200 μ g at 10 days) whereas no further decrease was recorded with noradrenaline. The high efficiency of the drug in depleting serotonin is illustrated in the spinal cord, where 10 μ g 5,7-DHT caused a near maximal depletion of 5-HT (85% reduction at 10 days). There was no significant influence on the spinal cord NA content at this dose. However, increasing doses of 5,7-DHT provoked a progressive reduction also of spinal cord NA concentrations (15% NA of control, 10 days after 200 μ g 5,7-DHT). The effect on spinal cord 5-HT was always greater than on NA.

As with 5,6-DHT, the reduction of 5-HT was different in individual brain regions. 10 days after 200 μ g 5,7-DHT, the reduction in 5-HT varied from about 55% in pons/medulla oblongata to 85% in the forebrain rest preparation and the septum. Generally, the depletion was higher in ventricle-near brain regions (septum, striatum, hypothalamus) and lower in the cell body rich regions of the rat CNS (mesencephalon and pons/medulla oblongata), similar to what has been found with 5,6-DHT.

Principally, the time-course pattern of depletion and recovery of serotonin after 5,7-DHT was similar to that after 5,6-DHT but two main differences were observed. The initial depletion of 5-HT measured at 3 hrs or 1 day, was very pronounced (between 65 and

80 %) in the septum, hypothalamus, mesencephalon and pons/medulla oblongata. In the same regions, a variable, partial recovery of 5-HT concentrations occurred within the subsequent 3 or 4 days. In all brain regions and the spinal cord, near-minimum levels were reached after 5,7-DHT by 10 days. A slight long-term recovery of serotonin between 10 and 45 days was noticed in mesencephalon and pons/medulla oblongata, regions known to comprise many serotonin cell bodies.

200 μ g 5,7-DHT caused a strong depletion of NA in whole brain and spinal cord (about 75% reduction at 3 hrs). By 4 days, the NA levels had partially recovered (45% of control in brain and 70% of control in spinal cord), indicating that the initial profound effect was probably due to a displacement of noradrenaline by a false transmitter like mechanism. The reduction of whole brain NA persisted up to the 45th day, whereas the spinal cord showed a second phase of NA depletion between 4 and 10 days (from about 70% of control at 4 days to about 15% at 10 days). In contrast, whole brain dopamine concentrations remained practically unchanged up to 10 days, but a slight, non-significant increase was found at 45 days (135% of control).

The profound, and long-lasting effect of 5,7-DHT on central serotonin neurons was confirmed in a study on regional tryptophan hydroxylase activity after administration of 150 μ g 5,7-DHT (Paper IV). Ventricle-near regions of rat brain (septum, striatum, hypothalamus and mesencephalic tectum) were substantially depleted already at 2 days and showed no further increase in the degree of depletion at 12 days. Mesencephalic tegmentum tryptophan hydroxylase was slightly depleted at 2 days (88,5% of control) and showed a further

reduction of activity at 12 days (to 39,5% of control). A similar, time-dependent increase in enzyme activity depletion between 2 and 12 days was unraveled in the forebrain rest preparation, pons/medulla oblongata and spinal cord. Since tryptophan hydroxylase is probably selectively confined to serotonin neurons in rat brain (Clineschmidt et al. 1971), the data on the reduction in enzyme activity at 12 days are consistent with the idea that 5,7-DHT acts by inducing a toxic disintegration of terminal and non-terminal axons whereas cell bodies are much less affected (the highest activity levels of tryptophan hydroxylase were found in the cell body rich regions pons/medulla oblongata and mesencephalic tegmentum after 5,7-DHT), similar to what has been found after 5,6-DHT.

Further support for the idea that 5,7-DHT acts toxic on central serotonin and noradrenaline neurons was gained by showing that incubated slices from cortex, hypothalamus and spinal cord of rats pretreated with one injection of 200 μ g 5,7-DHT (9-13 days prior to the incubation experiment) revealed a significant reduction in the ability to accumulate 3H-5-HT and 3H-NA (85-9% reduction in uptake of unmetabolized 3H-5-HT in all regions, 40-57% reduction in 3H-NA uptake, Paper VI).

All doses of 5,7-DHT investigated (25-300 μ g) caused a more or less rapid disappearance of yellow fluorescent indoleamine-containing terminals from ventricle-near regions of the brain (e.g. suprachiasmatic nucleus and subcommissural organ) and, partly also from ventricle-distant structures, such as the globus pallidus, the amygdaloid complex and the grey matter of the spinal cord (see Paper VI and Baumgarten

and Lachenmayer 1972). Following an initial phase of transmitter depletion, many NA terminal systems in the forebrain regained near-normal fluorescence by 2-4 days, but during the following days there was a gradual disappearance of NA fluorescence in terminals of the telencephalic cortex, suggesting a more or less selective toxic lesion to the dorsal norepinephrine pathway. The effect of 5,7-DHT on the ventricle-bordering NA terminal systems in the diencephalon was less pronounced.

By 24 hrs many dilated and distorted indoleamine- and catecholamine-containing non-terminal axons were found in the reticular formation of the mesencephalon, pons/medulla oblongata, in the anterior and lateral funiculus of the cervical spinal cord, in the preoptic region and the septum complex, and especially in the so called dorsal norepinephrine bundle at the level of the mesencephalon. While dopamine terminal systems remained largely unaffected by 5,7-DHT, some swollen green fluorescent catecholamine-containing axons developed after the higher doses of 5,7-DHT (150-300 μ g) in the olfactory tubercle, in the nucleus caudatus putamen and in the amygdaloid complex. Except for a transient swelling and disorganization of myelin, no long-term toxic effects on non-monoaminergic structures were unraveled in the electron microscope following 5,7-DHT application, in contrast to what has been documented after medium or high doses of 5,6-DHT. These findings point to a lower general toxicity of this meta-substituted dihydroxyindoleamine compared to the ortho-substituted compound 5,6-DHT, and, at the same time, point to a high intrinsic selective neurotoxicity against CNS 5-HT and NA neurons.

4.6 The effect of various tryptamine derivatives on the uptake of 3H-5-HT, 3H-NA and 3H-DA, studied in vitro (Paper VIII)

The differences in potency, side effects and specificity of action of 5,6- and 5,7-DHT suggest differences in the metabolic handling of both compounds by 5-HT and NA neurons, or even differences in the basic molecular mechanism of action of both substances. In order to understand at least part of these differences in the action of 5,6- and 5,7-DHT upon CNS monoamine neurons, the uptake affinity properties of these two dihydroxytryptamines and certain other di- and monohydroxylated tryptamines were investigated and compared (Paper VIII). Uptake affinity was measured in vitro by evaluating the inhibition of uptake of labelled 5-HT, NA or DA into synaptosome fractions of homogenates from hypothalamus (for 5-HT and NA uptake) or striatum (for DA uptake). Uptake affinity was regarded as an important parameter which determines at least partly the selectivity of actions of a given transmitter analogue against NA, DA or 5-HT neurons. The selectivity of action of any monoamine congener by all probability depends on its active uptake, concentration and retention by the different monoamine neurons. The uptake and accumulation of the neurotransmitter analogue is mediated by a membrane-bound, energy-dependent transport mechanism which has, at low concentrations of the amine, a high degree of selectivity for the natural substrate. Thus, e.g. exogenous 5-HT is preferentially taken up and accumulated by 5-HT neurons when given in vitro to slices of rat hypothalamus in concentrations of about $1 \times 10^{-7} \text{M}$ (Shaskan and Snyder 1970), despite the

presence of considerable amounts of NA and DA terminals in the same tissue. Since uptake occurs mainly into nerve terminals, it can be measured also in synaptosome fractions (Coyle and Snyder 1969) of homogenates from appropriate brain regions. In synaptosomes fractions, uptake is known to increase linearly at least during the first 5-10 minutes of incubation and, during this time period, little metabolism of the tritiated amine takes place. The advantage of synaptosome fractions over brain slices is that diffusion problems are much less important than in slices and that extra-neuronal uptake which might occur in slices can be neglected in synaptosome fractions.

For 5-HT uptake, the IC-50 values (concentration of indoleamine causing a 50% inhibition of the uptake of radioactive 5-HT) were measured which are approximately equal to the Ki values when a low concentration of amine is used. For NA and DA uptake, the analogues were tested at a fixed concentration at 1×10^{-6} or 1×10^{-5} M, using 1×10^{-7} M 3H-catecholamine. The results obtained reveal that of all the compounds tested, 5,6-DHT is the most selective inhibitor of 3H-5-HT uptake. 5,6-DHT is closely followed by 6-HT which has been suggested as a marker for serotonergic neurons by Jonsson et al. (1969). This is true only when uptake into DA containing neurons is blocked, or, preferably, when the interfering DA and even NA terminals are removed from the tissue by pretreatment with e.g. 6-OH-DA (see Jonsson et al. 1969). 4,5-DHT is provided with a rather good affinity to the 5-HT uptake sites, whereas 5,7-DHT is more than 6 times potent than 5,6-DHT. 7-HT competes for uptake into 5-HT neurons only slightly better than 5,7-DHT and both are more potent in

inhibiting 5-HT uptake than 4-HT and 6,7-DHT. Methylation of the 5 hydroxyl group in 4,5-DHT, which gives 4-hydroxy-5-methoxytryptamine, results in a more than 3 fold decrease in uptake affinity (see Baumgarten, Björklund, Horn and Schlossberger 1973). This compound which is highly interesting since it is the only monohydroxylated tryptamine that causes almost selective toxic damage to CNS 5-HT neurons (Baumgarten and Lachenmayer unpublished), inhibits the uptake of 3H-5-HT only very weakly.

Some important conclusions regarding the specificity of action and the molecular mechanism of action of toxic and non-toxic indoleamines can be drawn from these findings. The most specific compound with a K_i close to 5-HT itself is 5,6-DHT, which according to the theoretical considerations, was presumed to be the most favourable drug for chemical degeneration of serotonin neurons. Its high selectivity of action against CNS monoamine neurons is not only caused by its high affinity to the 5-HT uptake sites but also by its affinity for catecholamine transport being 7 times less than for 5-HT. This difference in affinity to the indoleamine and catecholamine uptake sites cannot, however, account for its surprising selectivity, since the amounts administered to experimental animals are so far in excess of the optimum substrate concentrations normally used by the two transport systems (1×10^{-8} - 1×10^{-8} M), that other factors have to be considered to explain the existing specificity of 5,6-DHT against 5-HT neurons.

These factors, still unexplored and unknown today, may be found in differences of metabolism of 5,6-DHT by indoleamine and catecholamine neurons. One possible

explanation would be that a significant fraction of 5,6-DHT taken up into catecholamine neurons is metabolized by catechol-o-methyltransferase and, thereby, transformed into an inert hydroxy-methoxytryptamine. It is well established that 5-HT is primarily metabolized to 5-HIAA by monoamine oxidase, and that o-methylation probably can be neglected in serotonin-containing neurons in contrast to NA ones. It should be pointed out that omethylation of 5,6-DHT at either the 5 or the 6 position has in fact been demonstrated by Axelrod and Lerner as early as 1963. Thus, the ideas put forward above to explain the differences in the effect of 5,6-DHT on CNS indoleamine and catecholamine neurons respectively have some support. From our own recent experiments (Baumgarten and Lachenmayer unpublished) we know that neither 5-hydroxy-6-methoxy- nor 5-methoxy-6-hydroxytryptamine induce degeneration in any type of monoamine containing neurons of rat brain after their intraventricular administration.

The same consideration probably is valid also what concerns 5,7-DHT which, according to the uptake affinity constants, should damage central NA neurons to a greater extent than central 5-HT neurons. When applied in doses higher than 50 μ g, however, the extent of degeneration in 5-HT neurons significantly exceeds that provoked in central NA neurons. Differences in the anatomical distribution of terminals or axons of both neuron systems in rat brain cannot solely account for the preferential action of 5,7-DHT on 5-HT axons and terminals, since both systems have a principally similar arrangement of their projections in brain and since 5,7-DHT is a remarkably stable

compound (when compared to 5,6-DHT) and can probably freely diffuse into brain matter from the ventricles or subarachnoid space and thus reach ventricle-distant terminals or axons of either system. Thus we are facing the interesting possibility that the action of the toxic neurotransmitter analogues may be individually modified by basic chemical or metabolic properties of the neurons affected. Studies on the metabolism of labelled 5,6- and 5,7-DHT will be necessary to clarify this interesting problem.

The results on uptake interference of various indoleamines with the 5-HT uptake also show that for optimum binding to the membrane transport system, the presence of a 5 hydroxyl group is an important prerequisite in the dihydroxytryptamines, as revealed e.g. by the significantly decreased uptake affinity of the 5-o-methylated analogue 4-hydroxy-5-methoxytryptamine in comparison to 4,5-DHT, or as noted by the comparatively low inhibition of 5-HT uptake by 6,7-DHT (see Baumgarten, Björklund, Horn and Schlossberger 1973). A catechol-like ortho arrangement of the hydroxyl groups in 5,6- and 4,5-DHT seems to favour uptake affinity whereas a meta substitution of the hydroxyl groups, realized in e.g. 5,7-DHT, decreases uptake affinity despite the presence of one of the OH groups in the optimum position (5 position).

An important observation is, that all tryptamine derivatives compete more or less well for uptake into noradrenaline or dopamine neurons, and the difference in binding affinity of a given compound for 5-HT uptake on one hand and NA or DA uptake on the other hand is sometimes rather small. This implies that most tryptamines in general have a rather good affinity to catecholamine uptake systems and are not as selective

for 5-HT uptake as catecholamine analogues are for NA uptake (e.g. 6-OH-DA, see Heikkila and Cohen 1973). Therefore, specific effects of tryptamines on 5-HT neurons can be expected only in a very restricted dose range and unselective side effects on catecholamine neurons are more likely to occur than with toxic catecholamine analogues at 5-HT neurons (e.g. no inhibition of uptake of 3H-5-HT by 6-OH-DA at 10^{-7} - 10^{-4} M, Heikkila and Cohen 1973).

A key to the understanding of the molecular mechanism of action of any neurotoxic monoamine derivative seems the observation that not only dihydroxytryptamines do cause degeneration but also a monohydroxytryptamine, namely 4-hydroxy-5-methoxytryptamine. Like most of the dihydroxytryptamines, also this monohydroxyderivative is very labile at biological pH conditions, and, as shown in test tube experiments, readily converts into a coloured quinoid compound. This implies that the toxicity may be connected to the quinoid or quinone metabolites rather than the amines themselves. Quinones are known to eagerly undergo addition or substitution reactions with many high or low molecular compounds, and it seems conceivable that the irreversible binding of these reactive quinones or quinone-like substances to structures that are important for the maintenance of the neuron's integrity can induce degeneration. Saner and Theenen (1971) were the first to show that long-lasting, probably irreversible binding of labelled 6-OH-DA or a metabolite to protein fractions of tissues does occur.

5 CONCLUSIONS

A. In two independent studies, the effect of intraventricularly applied 5,6-dihydroxytryptamine on CNS monoamine concentrations was analyzed in the rat. Doses from 25-100 μ g 5,6-DHT caused a long-lasting depletion of serotonin in nearly all regions investigated. 50 μ g 5,6-DHT did not bring about any quantitative changes in either brain NA or DA concentrations whereas 75 μ g 5,6-DHT induced a temporary mild drop in whole brain DA (between 1 and 4 days) and a transient significant elevation of whole brain NA between 4 and 30 days. The extent of 5-HT depletion was found to depend on both the type of anaesthesia and the speed of injection, and, in addition, to depend on the method of determination applied. The reduction in 5-HT levels caused by 5,6-DHT varied in different brain regions. It was most pronounced in the spinal cord and mesencephalon, significant in the ventricle-near brain regions (hypothalamus and septum) and moderate in the forebrain rest sample and pons/medulla oblongata as well as the striatum preparation. The differences in the pattern of time course of depletion of 5-HT noted in different brain regions suggest that 5,6-DHT acts in different ways on central serotonin neurons. In a properly selected dose range 5,6-DHT thus has remarkably selective effects on CNS 5-HT concentrations.

B. Long-term recovery patterns of 5-HT levels in selected brain regions and in spinal cord following one single injection of 75 μ g 5,6-DHT were analyzed in rats with a survival time of 1, 2, 3, 4 and 6 months. Serotonin concentrations were found to gradually rise in practically all brain regions between 10 days and half a year, except the spinal cord. Hypothalamus and septum were characterized by a steep increase in 5-HT levels, resulting in values significantly above control at 3 and 4

months. Serotonin levels in the striatum, mesencephalon and forebrain rest sample increased to values not differing from control ones by 6 months. The pons/medulla oblongata differed from the last mentioned regions in that the rise in 5-HT lead to supranormal values by 6 months after 5,6-DHT. The long-term recovery of 5-HT in all regions of the rat CNS, except the spinal cord, is compatible with the idea of significant regeneration of indoleamine neurons after preceding partial terminal and axonal degeneration induced by 5,6-DHT.

C. In a detailed fluorescence histochemical investigation, the short- and long-term effects of 25 and 75 μ g 5,6-DHT, injected into the lateral ventricle, were analyzed on the bulbo-spinal monoamine neuron systems in the rat. The primary site of action of 5,6-DHT was the non-terminal indoleamine axon, at a level of the lower medulla oblongata and most cranial spinal cord, which reacted to the drug by the development of highly fluorescent and distorted local dilatations. Terminals of 5-HT axons - innervating the anterior horn of the entire spinal cord - largely resisted the direct effects of 5,6-DHT and started to gradually loose their formaldehyde-induced fluorescence by 24 hrs, until they had completely disappeared by 10 days. This delay of the effect of 5,6-DHT indicates that the indoleamine terminals degenerate secondary to a direct toxic lesion of their non-terminal axons. As early as 6-10 days after 5,6-DHT, newly formed, delicate, highly fluorescent indoleamine axons were detected in regions comprising accumulations of drug distended non-terminal axons. These newly formed axonal sprouts continuously developed during the subsequent weeks growing into the surrounding brain tissue and finally reinnervated by means of newly formed terminals the

most cranial segments of the spinal cord's grey matter. This suggests successful reinnervation of chemically denervated regions of the rat CNS. Besides orderly directed growth, there occurred unorthodox sprouting of axons into parts of the brainstem not normally innervated by indoleamine fibres, indicating formation of aberrant connexions in the adult rat brain during axonal regeneration.

D. Proof for the idea that 5,6-DHT can in fact induce direct terminal degeneration of neurons in brain was obtained from careful electron microscopical investigations on brain regions known to receive indoleamine (subcommissural organ; periventricular hypothalamus) and indole- and catecholamine projections (septum, caudate nucleus). In between 6 hrs and 2 days after 5,6-DHT, synaptic terminal and non-synaptic varicose unmyelinated axons were found in all regions analyzed exhibiting signs of damage and/or disintegration. Dystrophic, electron dense remnants of axonal profiles were removed by glial cell phagocytosis. Besides lesions of unmyelinated axons, also toxic damage of myelinated fibres were encountered in the head of the left caudate nucleus, i.e. where the highest concentration of 5,6-DHT was reached during the injection. This finding calls for caution when using 5,6-DHT as a tool for behavioral or functional studies without proper control of unselective damage by histological techniques.

E. The effects of intraventricular injections of 10-200 μ g 5,7-dihydroxytryptamine on rat brain and spinal cord 5-HT, NA and DA concentrations were analyzed 10 days after drug application. In whole brain and spinal cord 10-200 μ g 5,7-DHT caused a dose-dependent reduction in 5-HT and NA levels, the drop of 5-HT being larger than of NA with doses higher than 50 μ g 5,7-DHT. Dopamine was not affected at all. The time-course of deple-

tion and recovery of 5-HT was studied in individual brain regions. Ventricle-near (septum, hypothalamus) and cell body rich regions (mesencephalon, pons medulla oblongata) responded to a single high dose of 5,7-DHT (200/ug) by an early, rapid and profound decline in 5-HT between 3 and 24 hrs. After a transient recovery period, serotonin dropped to near-minimum values at 10 days. Spinal cord, forebrain rest sample and striatum revealed a protracted, gradual loss of 5-HT, giving minimum levels only at 10 days. Whole brain NA concentrations showed an initial profound depletion, intermediate partial recovery at 4 days and a late drop to near-minimum levels at 10 days. The long-term depression of NA and 5-HT levels suggests that 5,7-DHT causes degeneration of noradrenaline and serotonin axons and terminals and this interpretation receives support from fluorescence histochemical data and from data on the depletion of tryptophan hydroxylase in discrete regions of the rat CNS, 2 and 12 days after 5,7-DHT.

F. The % inhibition of uptake of 3H-5-HT, 3H-NA and 3H-DA caused by various di- and monohydroxytryptamines was measured in synaptosome fractions of rat hypothalamus (for 5-HT and NA uptake) and striatum (for DA uptake). The data obtained reveal that from all compounds tested 5,6-DHT is the most selective and potent inhibitor for 5-HT uptake, whereas 5,7-DHT is 6 times less potent in interfering with 5-HT uptake than 5,6-DHT and inhibits NA uptake to a slightly larger extent than 5-HT uptake. Despite these differences, 5,6- and 5,7-DHT are, at a given dose, roughly equipotent against CNS 5-HT neurons, pointing to additional factors besides uptake affinity that influence the degree of selective neurotoxicity and potency.

5,6- and 5,7-DHT are considered as interesting tools for the investigation of the anatomy and function of CNS indoleamine-containing neurons, provided that selective and unselective toxicity are properly evaluated by chemical, histochemical and electron microscopical methods.

The present work was carried out in the Department of Histology, University of Lund, Sweden, in the Department of Neuroanatomy, University of Hamburg, G.F.R., in the Department of Pharmacology, MRC Neurochemical Pharmacology Unit, Cambridge, England, in the Department of Pharmacology, ARC Institute of Animal Physiology, Babraham-Cambridge, England, and in the Department of Biochemical Pharmacology, National Heart and Lung Institute, Bethesda, Maryland, USA.

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